MICROBIOLOGICAL CHARACTERISTICS OF STORMWATER RUNOFFS AT EAST YORK (TORONTO) AND GUELPH SEPARATE STORM SEWERS

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Ministry of the Environment

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ABSTRACT

A six-month study was conducted to determine the microbiological characteristics of stormwater runoffs at East York (Toronto) and Guelph separate storm sewers. The results indicated that stormwater discharges from these two systems contain high levels of pollution indicator bacteria and some pathogenic bacteria. The pollution appears to be predominately of non-human origin and is mainly derived from animal wastes. In general, the runoffs resembled dilute raw wastewaters in microbiological composition and represent a public health risk.

The results of this study also showed the seriousness of urban stormwater runoffs as a major factor in "nonpoint" source pollution of surface waters.

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CONCLUSIONS

1. Discharges from the Barrington Avenue (Toronto) and the Woodlawn Road (Guelph) separate storm sewer systems contained significant quantities of fecal pollution indicator bacteria. The total coliform densities ranged from 3,000 to 1,190,000 per 100 ml. Fecal coliform levels varied from 200 to 560,000 per 100 ml. Fecal streptococcus densities, which were generally equal to or greater than fecal coliforms, ranged betweeen 3,000 and 620,000 per 100 ml.

2. Remarkably high populations of fungi were found in stormwater discharges at both sites. The concentrations fluctuated from 140,000 to 12,000,000 per 100 ml.

3. Opportunistic pathogenic bacteria viz. Pseudomonas aeruginosa and Staphylococcus aureus were infrequently isolated from runoffs at both storm sewers. The densities of these two organisms varied from 1 to 630 and 1 to 100 per 100 ml, respectively.

4. During this study, salmonellae were consistently detected in discharges from the Toronto and Guelph storm sewers. In several instances, these pathogens were readily isolated from as little as 10 ml aliquots of stormwaters. The salmonellae isolates belonged to four different serotypes including S. haardt, S. saint-paul, S. tennessee and S. typhimurium.

5. The strikingly high concentrations of microbial populations found in sediments from these two systems indicate that such sediments constitute a reservoir for various microorganisms which ultimately find their way into stormwater runoffs.

6. No typical patterns of the distribution of indicator bacteria or of pathogenic bacteria in stormwater runoffs could be established during this study. The peak microbial populations occurred either at the initial stages (0 to 15

minutes), middle (20 to 40 minutes) or the tail-end (45 to 60 minutes) of the sampling period of different storms monitored. The unpredictable and intermittent release of microorganisms in stormwaters suggests that any effect of "Initial Flushing" on microbiological quality of the discharges was minimal during an individual storm.

7. The Barrington Avenue storm sewer showed higher levels of microbial populations and yielded greater number of pathogenic bacteria than the Woodlawn Road system. In addition, some seasonal differences in the bacterial densitites of pollution indicator bacteria were noted at the Toronto site.

8. The data indicate that fecal pollution in these two separate storm sewers is predominately of non-human origin. Fecal material from animals and birds washed from the streets during rain storms appear to be the major source of pollution. In addition, runoffs from vegetation and soil may also contribute fluctuating levels of indicator bacteria to stormwater discharges.

9. The levels of microbial populations in stormwater runoff were strikingly high throughout the entire sampling period and many times approached densities found in raw sanitary wastewaters and therefore constitute a health hazard. The implied public health risk is substantiated by the recovery of pathogenic and potentially pathogenic bacteria in discharges from these two storm sewers. All four Salmonella serotypes found in this study have also been isolated from humans in Canada during recent years.

10. This study shows that the discharges from separate storm sewers can be a major source of pollution to receiving surface waters and reservoirs used for recreational purposes. Definite remedial measures must be applied to prevent public health risks and deterioration of surface water quality.

1.0 INTRODUCTION

During recent years there has been a growing recognition of the importance of stormwater runoff as a major source of pollution in natural surface waters. Seeveral studies have been conducted to determine the quantitative and qualitiative characteristics of stormwater discharges and their impact on receiving waters (1-16).

Weibel et al (2) reported that ninety percent of all stormwater samples from a separately sewered urban area had coliform, fecal coliform and fecal streptococcus counts exceeding 2900, 500 and 4900 per 100 ml, respectively. In ten percent of the samples the levels of these groups were greater than 460000, 76000 and 110000, respectively.

Burm and Vaughan (5) found that the total coliform levels in the runoff from separate storm sewers were about one tenth of those in combined sewers where median monthly values were as high as 37,000,000 per 100 ml. Fecal coliform densities in separate storm sewer runoffs were rarely more than twenty percent of the total coliform population. Also, the combined sewer discharges contained forty times as many fecal coliforms as were detected in separate storm sewers. They found that in both types of sewer systems fecal streptococcus densities were similar and were generally equal to or greater than the fecal coliform counts. Similarity, Benzie and Courchaine (6) found that the discharges from a combined sewer contained considerably larger quantities of total coliforms, fecal coliforms and fecal streptococci than those from a separate storm sewer system.

Burm (7) showed that total coliform, fecal coliform and fecal streptococcus densities in a large amount of the receiving water increased considerably as a result of overflows from combined sewers after a moderate rainstorm. In receiving waters located above the overflows, however, the effects

of the storm were negligible. He indicated that the relative increases in bacterial densities were dependent on the distance between the outfall and the sampling points. With the increasing distance from the discharge point, the increases in bacterial levels became less pronounced.

Geldreich et al (10) reported similarities in bacteriological composition between stormwater runoff from cultivated farms and runoffs from city streets, a suburban business district storm drain, and a wooded hillside. Strator et al (12) showed that runoff from street surfaces was heavily contaminated and in many respects resembled sanitary wastewater. Using calculations based on a hypothetical city, they indicated that the runoff from the first hour of a moderate to heavy storm (1.27 cm/hr) would contribute a substantially greater pollution load than would the same city's sanitary waste during the same event.

In addition to fecal pollution indicator bacteria, the presence of pathogenic bacteria has also been reported in stormwater. Evans et al (9) demonstrated the existence of a potential health hazard by isolating Salmonella thompson (at a level of 4,500/100 ml) in a stormwater sample from an urban business district separate storm sewer. This stormwater sample contained 3,800,000 total coliforms, 450,000 fecal coliforms, and 370,000 fecal streptococci per 100 ml.

The fecal pollution bacteria in stormwater runoff from the separate storm sewers have been shown to be primarily of non-human origin, while those in combined sewers are of human origin (2, 5, 6, 9, 10). The non-human fecal contamination in separate stormwater systems is mainly derived from fecal discharges of rodents, cats, dogs and birds (10).

Since "rainwater" contains insignificant levels of bacteria, its major contamination occurs on contact with the land environment, creating a potential pollution problem in the resulting runoff (10). Among the many and different sources of pollution in urban stormwater runoff are: street debris and litter, soil-

chemical mixtures washed from surrounding open lands, garden fertilizers, herbicides and soil conditioners, air pollutants, deicing chemicals, dirt, oil, grease and other contaminants washed from automobiles, combustion byproducts and animal waste (10, 12, 17, 18, 19, 20, 21, 22, 23).

Relatively little reported data are available on the microbiological quality of stormwater runoff in Canada. As a part of the Canada Ontario Agreement (CAO), a study was designed to provide such information by examining several storm sewers, in the Central Region of Ontario, Canada. The main objectives of this investigation were: (i) to determine the incidence and distribution of pollution indicator microorganisms and selected pathogens in runoff from storms of different intensities and duration at two separate storm sewers, (ii) to determine the probable sources of contamination, and (iii) to evaluate the significance of stormwater pollution and associated potential health hazards.

2.0 STUDY AREAS

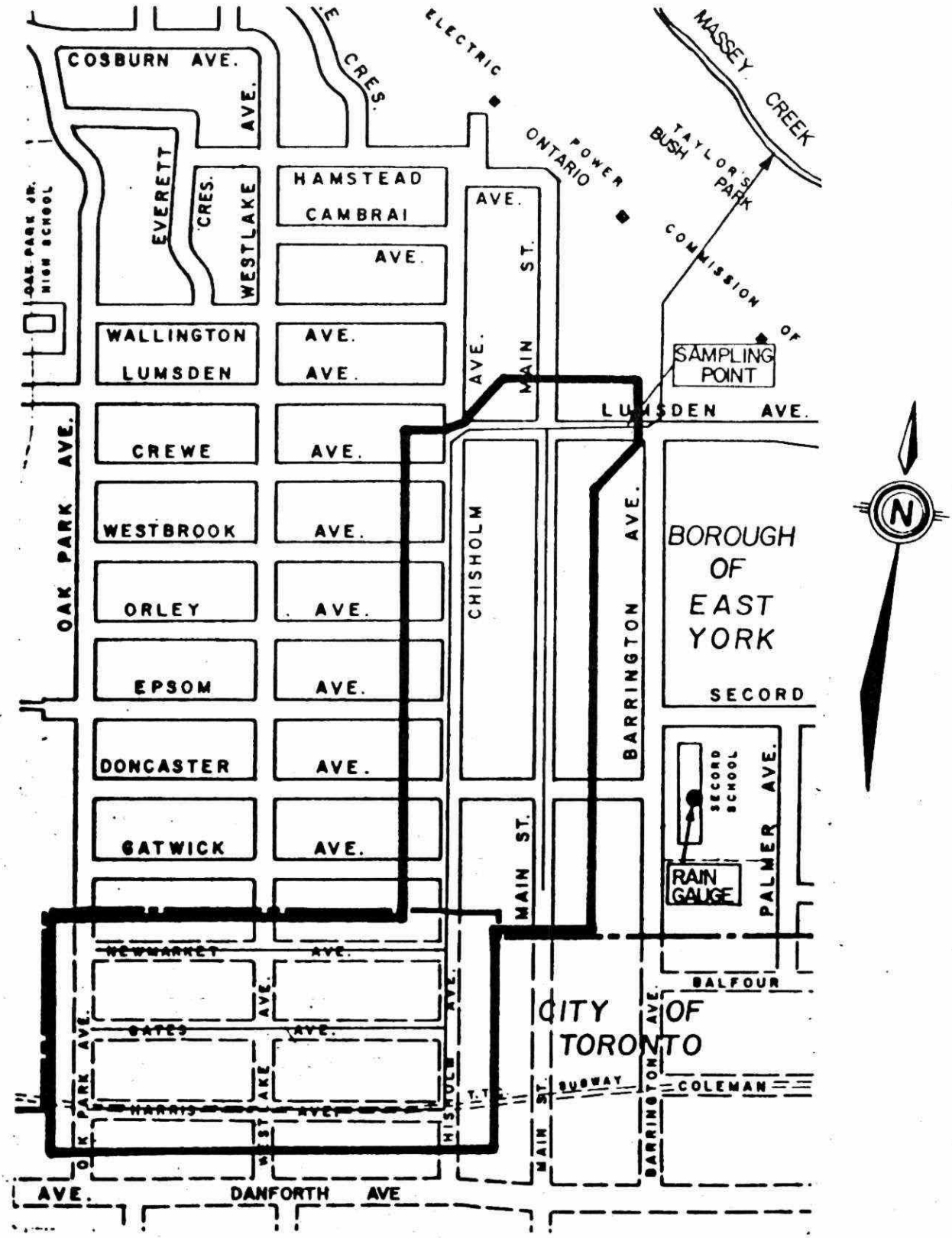
The detailed descriptions of the two stormwater runoff producing drainage area examined in this study are given below.

2.1 BARRINGTON AVENUE (EAST YORK), TORONTO

The Barrington site is located in the Borough of East York and includes part of the City of Toronto (Figure 1). With the exception of five or six corner stores, the area is entirely composed of single family residential housing ranging from 50 to 70 years in age. The site is 100% separate sewers with a total drainage area of 22.7 hectares (56.0 acres). The area containing buildings not connected to the storm sewer system is approximately 5.3 hectares (13.0 acres). Private lawns, walkways, driveways, roads and public sidewalks constitute the net area (17.4 hectares, 43.0 acres) contributing to stormwater runoff. The Chisholm Avenue and Main Street storm sewers flow to the trunk sewer on Lumsden Avenue, which in turn carries the flow to a 10 meter dropshaft at the end of Barrington Avenue and eventually northward to Massey Creek through a 152 cm circular pipe (Figure 1).

FIGURE 1. BARRINGTON DRAINAGE AREA

SCALE : 1 centimetre = 60 metres



2.2 WOODLAWN ROAD, GUELPH

The Woodlawn Road site is approximately 70.8 hectares (177.0 acres) and is located near the Speed River at the northwest suburban area of the City of Guelph at 80^{o} - 16' longitude and 43^{o} - 34' latitude. It is comprised almost entirely of new single and multiple family housing. Some commercial sites are also present in the drainage area. The area has 100% separate storm sewers.

3.0 MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

At both sites, water samples were collected manually in presterilized glass sampling bottles (ca. 170 ml) every five minutes for a period of one hour after the onset of a rainfall event. Four storm events were monitored at the East York Barrington outfall, whereas at the Guelph Woodlawn outfall only one event was examined.

Sediment samples (two at East York and one at Guelph) were collected by hand in sterilized Nalgene wide mouth jars (ca. 125 ml) on days when no event occurred.

Moore swabs, as modified by Spino (24) and Dutka and Bell (25), were installed in each storm sewer before the start of a rainfall event. Each swab was made of eight double layers of gauze folded to form a 20 x 35 cm pad. The pad was then cut into five 3-5 cm wide strips, which were gathered together at one end to form a 5 cm handle. The handle of the pad was attached to a 30 x 30 cm square of chicken wire; and each strip was spread and separately tied with string to the wire mesh so as to expose maximum surface area to the water. The pad and wire frame were wrapped in Kraft paper and sterilized by autoclaving at $121^{o}C$ for 15 minutes. In the field, the swab was unwrapped, and suspended by tying one end to a bouy with a 1 meter long rope and by attaching (with an other rope) the other end to a supporting structure.

Moore swabs were exposed to stormwater throughout the entire sampling period during each rainfall event. After exposure, the swabs were retrieved from the sampling site, drained for a few minutes, and placed in large sterile plastic bags.

All More swabs, water and sediment samples were transported on ice to the laboratory and were analyzed within 24 hours of collection.

3.2 MICROBIOLOGICAL ANALYSES

In all microbiological analyses where the Membrane Filtration (MF) technique was employed, Millipore 47 mm, 0.45 µm, white, gridded, ethylene oxide sterilized membrane filters (HAWG 04700) were used. All agar, broth and enrichment media used in microbiological determinations were Bacto brand, supplied by Difco Laboratories, Detroit, Michigan, U.S.A. Exceptions from the above are separately specified.

3.2.1 Pollution Indicator Bacteria

3.2.1.1 Stormwater Runoff

Water samples were analyzed for Total Coliform (TC), Fecal Coliform (FC) and Fecal Streptococcus (FS) using the Membrane Filtration technique. The procedures used for the estimation of these pollution indicator bacteria are described in detail in the Ministry of the Environment's "Handbook of Analytical Methods For Environmental Samples", Volume two (26). Therefore, only a brief account of these methods is given below.

Total coliform densities were determined on m-Endo agar Les plates after incubation at $35 \pm 0.5^{o}C$ for 22 ± 2 h. All colonies with a dull to bright, metallic, green-gold sheen were considered members of the coliform group. Colony counts were made and the densitites were expressed as coliforms per 100 ml sample. All red, pink, blue, white or colourless colonies lacking sheen were considered non-coliforms and were recorded as "Background" count per 100 ml sample.

The MacConkey membrane broth plates were used for fecal coliform determinations following incubation (in a floating plastic "cakette") at 44.5 ± 0.5°C in a circulating waterbath for 20 ± 1 h. All yellow, yellow-brown, and yellow-green colonies were counted as fecal coliform colonies. Densities were recorded as fecal coliforms per 100 ml sample.

Fecal streptococci were determined using m-Enterococcus agar plates incubated at 35 ± 0.5°C for 48 ± 3 h. All red, maroon or pink colonies were counted as fecal streptococci. Counts were expressed as fecal streptococci per 100 ml sample.

3.2.1.2 Sediment

The MF technique (as described above) and the most probable number (MPN) technique, following American Public Health Association Standard Methods (27), were used for the enumeration of pollution indicator bacteria in sediments. For both methods, a 1/10 sediment dilution was prepared by mixing 50 g (wet wt.) sediment in 450 ml buffered water dilution blank (26) in a Waring blender for 1 minute. From this suspension, further 10-fold dilutions were prepared using buffered water as required for various analyses.

TC were determined using a three dilution, five tube replication of lactose broth in a Standard MPN series. After incubation at 35°C for 48 h, lactose broth cultures positive for gas were inoculated into brilliant green lactose bile broth tubes and incubated at 35°C for an additional 48 h. All tubes showing acid and gas production were recorded as confirmed TC for the purpose of the MPN computation (MPN/100 ml).

For FC determinations, positive lactose broth cultures were used to inoculate EC broth tubes which were incubated at 44.5± 0.5°C for 24 h. Tubes showing growth and gas prodcution were recorded as confirmed FC and were included in the final MPN index of FC.

Fecal streptococci MPN were estimated using a five tube, three dilution sodium azide dextrose broth series. Inoculated tubes were incubated at 35°C for 48 h. All cultures showing growth were transferred into ethyl violet azide broth and were then incubated at 35°C for an additional 48 h. Ethyl violet tubes showing turbidity and a purple pellet at the bottom of the tube were recorded as confirmed FS.

3.2.2 Pseudomonas aeruginosa

P. aeruginosa densities in water samples were estimated on mPA agar medium using MF technique as described by Levin and Cabelli (28). After filtration of appropriate volumes of samples, membranes were transferred to mPA plates and incubated at 41.5 ± 0.5°C for 48 h. All flat colonies (0.8 to 2.2 mm in diameter) with tan, brown, greenish-black centers and pale outer rims were counted, and the densities were expressed as P. aeruginosa per 100 ml sample.

P. aeruginosa levels in sediments were enumerated by the MF procedure (as described above) and the MPN technique (27) using a three dilution, five tube series of modified Drake's medium 10 (29). The medium consisted of asparagine, 2.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_20$, 0.5 g; K_2SO_4, 10.0 g; glycerol, 10.0 g per liter of distilled water. The tubes were incubated at 41.5 ± 0.5°C for 48-96 h, examined under long-wave ultraviolet light and those tubes showing greenish fluorescent pigment were interpreted as presumptive positive. All presumptive positive tubes were confirmed by streaking on modified Christensen's acetamide agar and skim milk agar. The acetamide agar contained acetamide, 10.0 g; NaCl, 5.0 g; K_2HPO_4, 1.4 g; KH_2PO_4, 0.7 g; $MgSO_4.7H_20$, 1.54 g; phenol red, 0.012 g; agar, 15.0 g per liter of distilled water. The milk agar was prepared by dissolving 100.0 g skim milk (Difco) and 15.0 g agar in two separate aliquots of 500 ml distilled water. These two mixtures were sterilized separately and were mixed prior to pouring into petri dishes.

Milk agar plates incubated at 35°C for 48 - 96 h, were observed for casein hydrolysis and production of greenish fluorescent pigment. Acetamide agar plates were incubated at 41.5 ± 0.5°C for 48 - 96 h. Orange-red coloration around the colonies was considered as positive confirmation for P. aeruginosa MPN computation.

3.2.3 Coagulase-positive Staphylococci (Staphylococcuss aureus)

S. aureus densities in water samples were determined by MF technique using modified Baird-Parker's medium (30, 31). The medium was prepared by dissolving 63.0 g of Baird-Parker's medium (Oxoid) in 1000 ml distilled water and sterilized by autoclaving at 121°C for 15 minutes. To the medium cooled to 50°C, the following compounds were added aseptically: sulphamezethine (Matheson, Coleman and Bell), 5 mg/100 ml; actidione (Upjohn Company), 150 mg/100 ml; concentrated egg yolk-tellurite emulsion (Oxoid), 5 ml/100 ml. All ingredients were mixed thoroughly and the medium was poured into sterile petri plates which were used within 48 h of preparation.

Following filtration of appropriate volumes of water samples, membrane filters were placed on the modified Baird-Parker's medium plates and incubated at 35 ± 0.5°C for 24 - 48 h. All black, shiny, circular and convex colonies with a 2 - 5 mm clear zone (underneath the membrane filter) were scored as presumptive coagulase-positive staphylococci. All such colonies were streaked on Trypticase soy agar (BBL), incubated at 35 ± 0.5°C for 24 h, and subjected to Gram Stain and coagulase-mannitol tests. The latter test was peformed by inoculating tubes containing approximately 3.3 ml coagulase mannitol broth (BBL). For comparison, positive and negative control tubes were also used. All tubes were incubated at 35 ± 0.5°C for 18 - 24 h. Tubes showing coagulation (clot formation) and mannitol fermentation (production of yellow colour) were recorded as positive confirmation for coagulase-positive staphylococci (S. aureaus); the densities were expressed as per 100 ml sample.

3.2.4 Salmonella

For the isolation and identification of salmonellae, procedures outlined in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments" (32) were used with few modifications. The methods used in this study are summarized in Figure 2, and are described in detail in the following sections.

3.2.4.1 Stormwater Runoff

Stormwater samples collected every five minutes during the October 1, 1975 (East York outfall) event were analyzed individually. Water samples collected every five minutes during all other storm events were combined and analyzed as five (A, B, C, D and E) 'Composite Samples'. The protocol used to pool water samples is described in the Results section.

Water samples in variable aliquots (ranging from 10 to 400 ml) were filtered through 47 mm sterilized fiberglass prefilters (Sartorius SM 13400, British Drug House, Toronto, Ontario). The filters were aseptically placed in corresponding equal volumes of tetrathionate and selenite broths prewarmed to 41.5°C. These enrichment broths were incubated at 41.5°C for 20 ± 2 h, and a loopful of each broth was steaked on Brilliant Green (BG) agar and on Xylose Lysine Deoxycholate (XLD) agar plates. All plates were incubated at 41.5°C for 20 ± 2 h. After incubation, colonies appearing to be salmonellae* were streaked on MacConkey agar plates and also inoculated into O-nitrophenol β-D-galactopyranoside (BDH) - phenylalanine agar (ONPG-PA) tubes, which in turn were incubated overnight (20 ± 2 h) at 35°C. Lactose negative (MacConkey agar) and ONPG-PA negative colonies were inoculated into tubes of Motility Sulfide Medium (MSM), Triple Sugar Iron (TSI) agar and H broth. Presumptive Salmonella colonies growing on MacConkey agar plates were used as the source of inoculum for these tests. After incubation at 35°C for 20 ± 2 h, reactions on TSI and MSM were recorded. The MSM cultures

* Pink-opaque colonies on BG and pink-red, shiny black centered colonies on XLD.

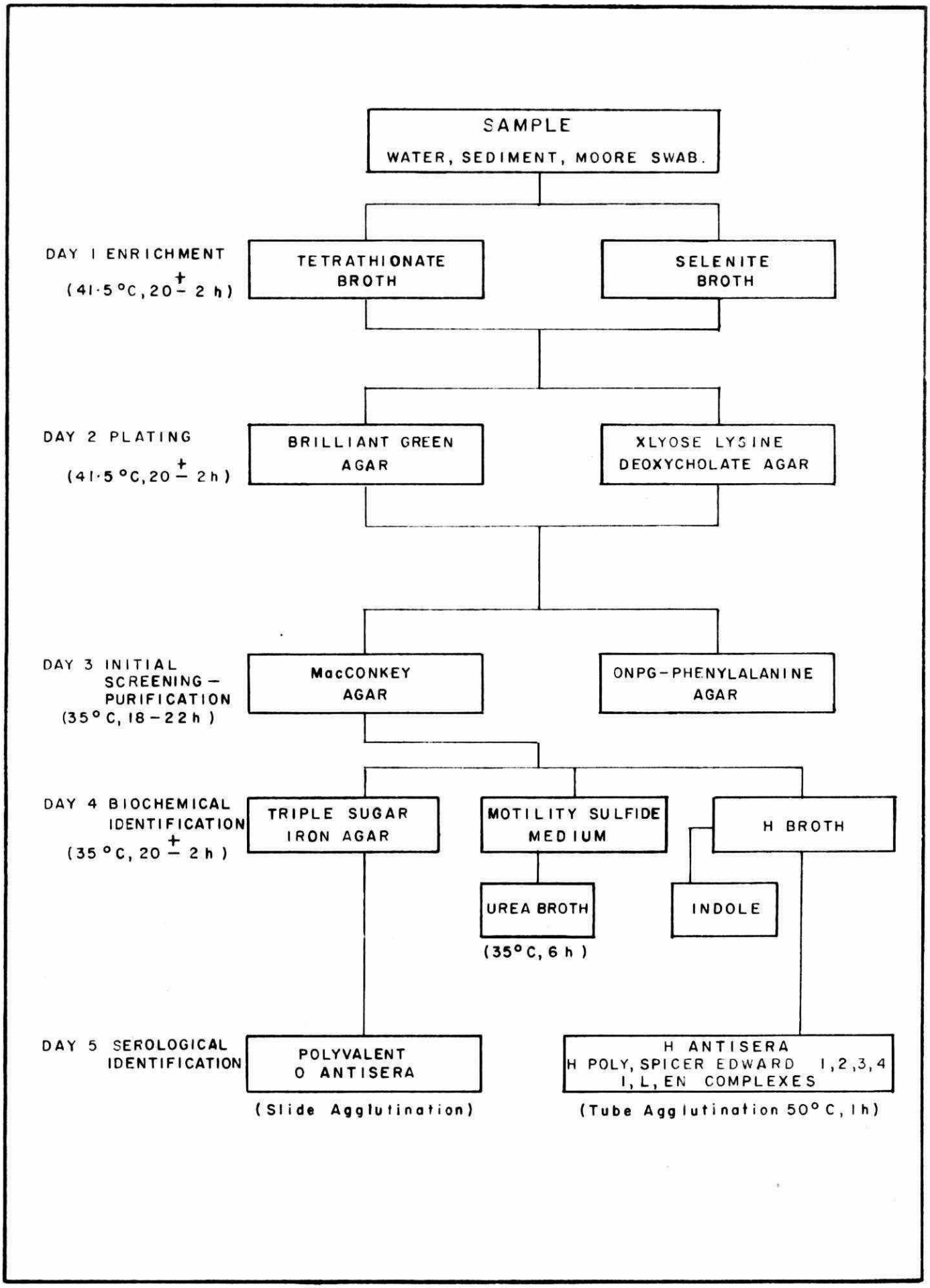


FIGURE 2: SCHEME FOR SALMONELLA ISOLATION AND IDENTIFICATION

were then overlayed with 1.0 ml of urea broth and incubated at 35°C for approximately 6 h to determine urease activity. A 3.0 ml aliquot of H broth was added to an equal volume of 0.6 N formyl saline, and a 2.0 ml aliquot was tested for indole production with Kovac's reagent. Cultures giving reactions typical for Salmonella in these media were biochemically and serologically identified by the methods of Edwards and Ewing (33).

Cultures that produced alkaline slants, acid butt, and gas, with or without H_2S in TSI, and which exhibited motility and H_2S production in MSM, and which were also urease negative were subjected to serological testing. All cultures were first tested for Salmonella O (somatic) antigens by means of slide agglutination with Salmonella polyvalent O antisera using growth on TSI slants. Cultures showing a positive reaction were then tested for Salmonella H (flagellar) antigens by means of multiple tube agglutination test. Approximately 0.5 ml of formalin-treated H broth was added to 0.5 ml of 1:200 dilution of the following Salmonella H antisera: H poly, Spicer Edward 1:4, EN Complex, I Complex and L Complex. All tubes for H antigens were incubated for 1 h at 50°C in a water bath. Cultures confirming to this stage as Salmonella were sent to the Ontario Ministry of Health, Enteric Reference Center, Rexdale, Ontario, for confirmation and identification of Salmonella serotypes.

3.2.4.2 Moore Swab

The five strips of each Moore swab were aseptically separated with sterile scissors and forceps. Three strips were transferred aseptically to separate 450 ml capacity wide mouthed glass bottles containing 300 ml of tetrathionate broth prewarmed to 41.5°C. The remaining two strips were each placed aseptically in 300 ml of prewarmed (at 41.5°C) selenite broth. The incubation and further processing of Moore swab enrichment broths for Salmonella isolation was similar to the methods described above for water samples.

3.2.4.3 Sediment

The membrane filter and direct inoculation techniques were used for the isolation of Salmonella from sediments. One ml aliquots of ten-fold sediment dilutions (preparation method described under the pollution indicator bacteria section) were filtered through membrane filters. The membranes were aseptically transferred to prewarmed (at 41.5°C) 10 ml volumes of tetrathionate and selenite enrichment broths. In addition, 1, 10, 50 or 100 ml aliquots of 1/10 sediment suspension were directly inoculated into appropriate volumes (1:10, 10 and 50:50, 100:100) of tetrathionate and selenite broths prewarmed to 41.5°C.

Twenty-five gram aliquots of one sediment sample (from East York) were directly placed into 300 ml volumes of prewarmed tetrathionate and selenite broths. All enrichment broths were incubated and further analyzed for Salmonella isolation following the procedures outlined above for water samples.

3.2.5 Total Fungi (Molds and Yeasts)

Fungal densities both in water and sediments were estimated by the spread plate (SP) technique using Streptomycin Terramycin Malt Extract Agar (STMEA) medium. This medium, which contained 30.0 g Malt extract, 5.0 g peptone and 15.0 g agar, was sterilized by autoclaving at 121°C for 15 minutes. Membrane filter sterilized solutions of streptomycin (Sigma Chemical Company, Saint Louis, Missouri) and terramycin (Pfizer Company Ltd., Montreal, Quebec) were separately added to the cooled autoclaved medium to obtain a final concentration (of each antibiotic) of 70 µg/ml.

Sample and/or sample dilutions of water and sediment (prepared as described in Section 3.2.1) were plated in one ml aliquots onto STMEA plates. The plates were predried, with covers removed, in a filtered air laminar flow hood at room temperature for 1 h. For plating, the Fisher Rota-Plate Inoculating Turntable (Fisher Scientific Company, Toronto, Ontario) was used to obtain a uniform distribution of the sample. The inoculated plates were allowed to dry on the

laboratory bench, inverted and then incubated (in a plastic cakette) at 20°C for 7 days. The developing colonies of both yeast and molds (total fungi) were counted with the aid of a Quebec Colony Counter. Fungal densities were expressed as colony-forming units (CFU) per 100 ml water sample and CFU per gram sediment sample.

4.0 RESULTS

4.1 BARRINGTON AVENUE (EAST YORK), TORONTO

4.1.1 Stormwater Runoff

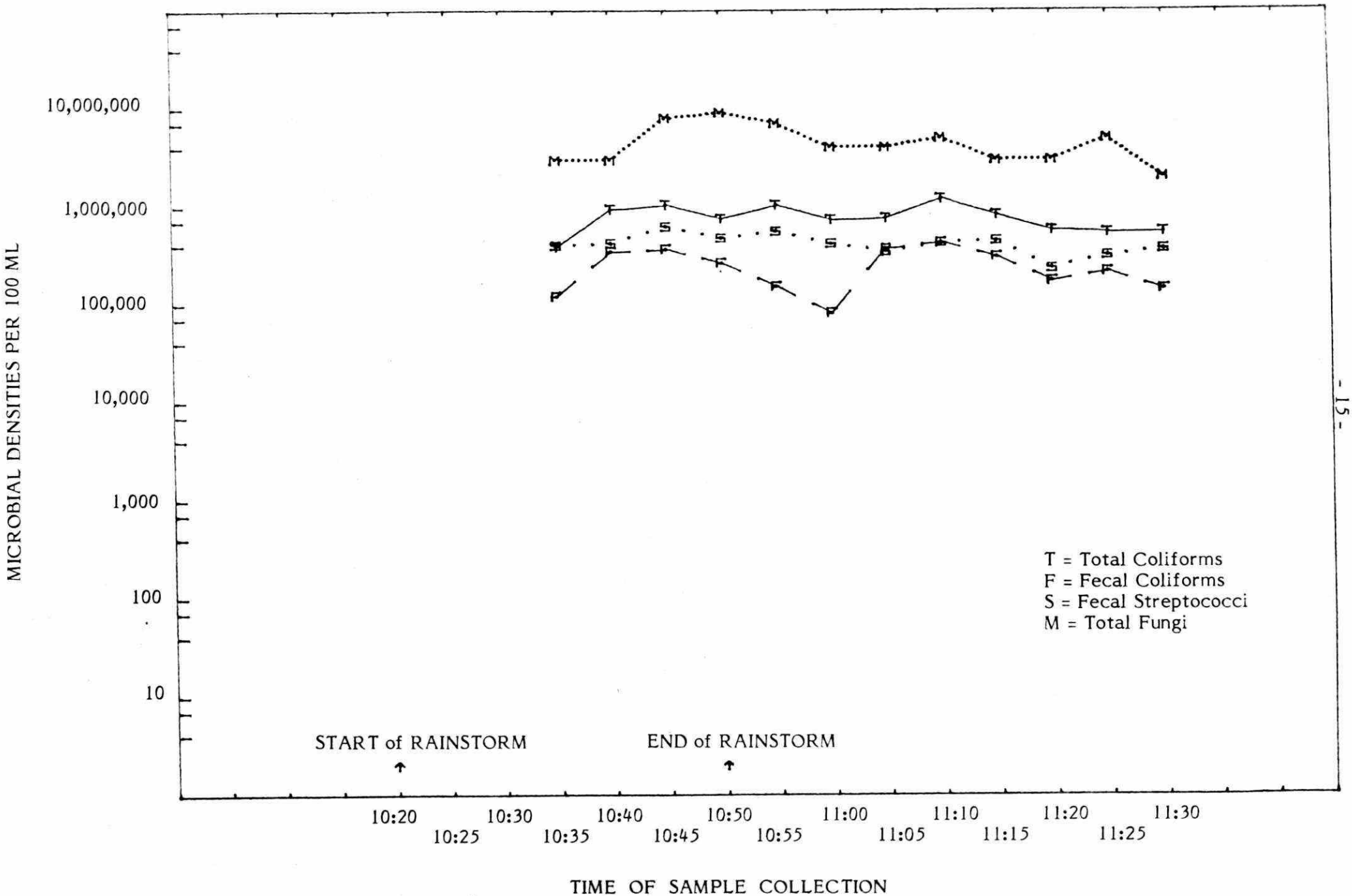
Microbiological data obtained from stormwater samples collected during four storm events are presented in Appendix I-IV. Due to the uniqueness and diverse nature of all storm events, their results are described individually.

Storm of October 1, 1975

A total of 2.3 mm rain fell during the storm which lasted for 30 minutes. Sampling was initiated 15 minutes after the onset of the storm and continued for 40 minutes after the end of the storm (Appendix I).

The densities of pollution indicator bacteria in stormwater samples are shown in Figure 3. Total Coliform (TC) levels showed an oscillating pattern of distribution. Initial counts (380,000/100 ml) exhibited a generally rising trend with the increasing time of sampling for up to 20 minutes. The densities then decreased slightly only to peak again in the 35 minute sample. Thereafter, counts declined to around 530,000/100 ml in the 45 to 55 minute samples. Fecal Coliform (FC) densities ranged from 120,000 to 420,000/100 ml and showed a distribution pattern similar to that of TC. The highest FC count was recorded in the 35 minute sample, while in the 0 and 55 minute samples the densities were 120,000 and 140,000/100 ml, respectively. Fecal streptococcus (FS) levels varied from 230,000 to 620,000/100 ml, and unlike TC and FC, showed relatively uniform distribution pattern. The maximum FS count was noted in the 10 minute sample, whereas the

FIGURE 3. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORMWATER RUNOFFS, October 1, 1975, Barrington Avenue (East York), Toronto



lowest count was obtained in the 45 minute sample. In all samples, the FS densities were generally much higher than those of FC.

The initial low counts of total fungi (3,000,000/100 ml) also exhibited a generally rising trend to reach the maximum level (9,000,000/100 ml) in the 15 minute sample (Figure 3). Thereafter, fungal densities declined steadily and the lowest counts were observed in the 55 minute sample.

S. aureus was detected in only seven samples and the densities were generally low ranging from 12 to 40/100 ml (Appendix 1).

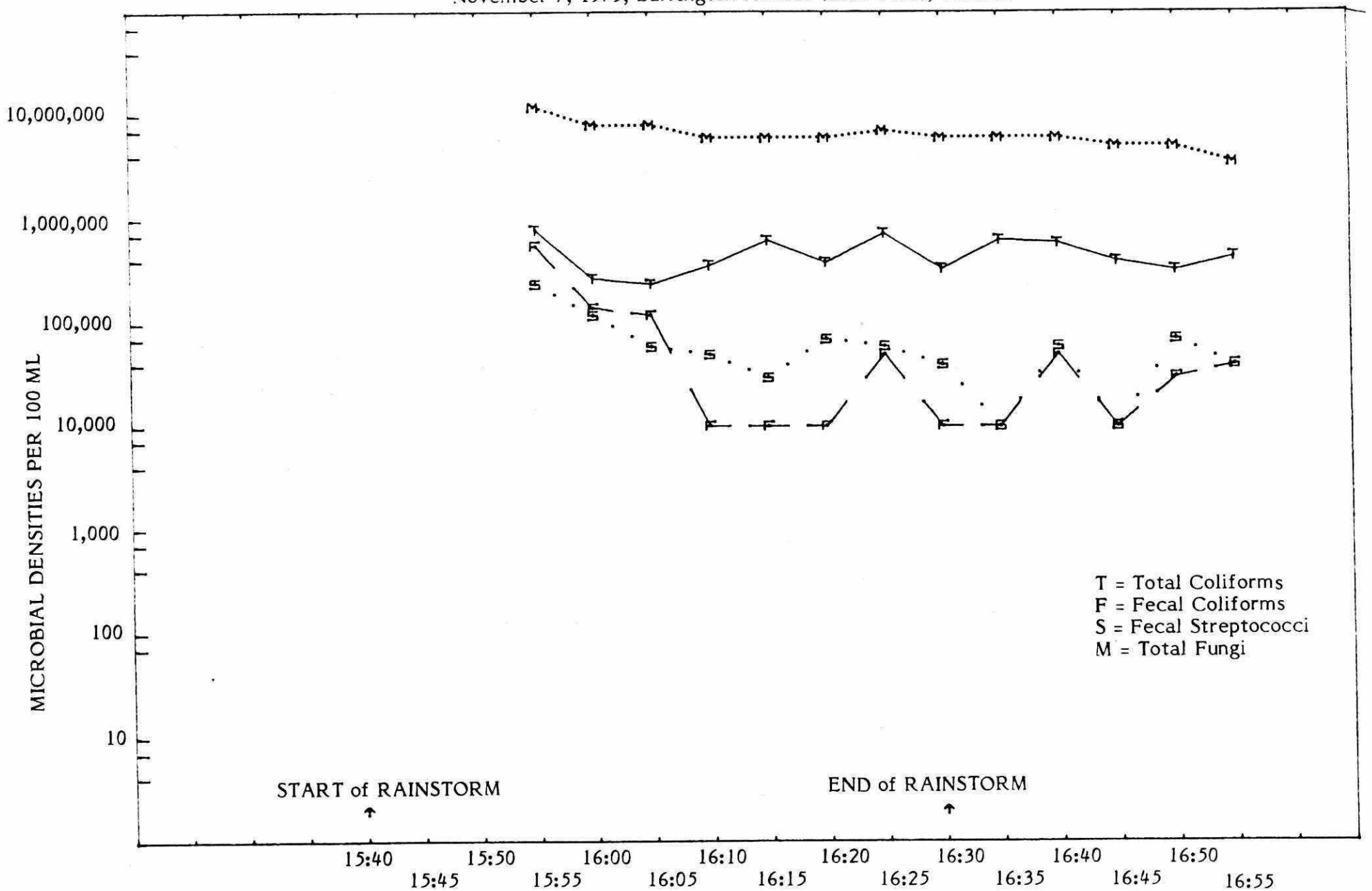
Salmonellae were isolated only from the 15, 20, 25 and 30 minute samples (Appendix 1). Two Salmonella serotypes, including S. typhimurium var. copenhagen and S. saint-paul, were isolated from as little as 10 ml of the water samples using tetrathionate-brilliant green media combination. No salmonellae were isolated from the Moore swab during this storm event.

Storm of November 7, 1975

Only 1.0 mm rainfall accumulated during the storm event which was of 50 minutes duration. Sampling began 15 minutes after the initiation of the storm and was completed 25 minutes after the end of the storm event (Appendix II).

The levels and the distribution of the TC , FC and FS in stormwater samples are given in Figure 4. The peak populations of these bacteria were observed in the first (0 minute) sample which was in fact collected 15 minutes after the commencement of the storm. Thereafter, total and fecal coliform densities decreased and increased with no consistent pattern of distribution. TC counts ranged from 240,000 to 790,000/100 ml, and FC levels ranged from 10,000 to 550,000/100 ml (Appendix II). After the initial peak, FS densities declined steadily but showed a relatively uniform pattern of distribution. The minimum and maximum values for FS were 10,000 and 240,000/100 ml, respectively. Furthermore, with few exceptions, FS densities were consistently higher than FC in stormwater runoff samples.

FIGURE 4. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORMWATER RUNOFFS, November 7, 1975, Barrington Avenue (East York), Toronto



The maximum levels of fungi (12,000,000/100 ml) were found in the 0 minute (first) sample. In general, fungal densities decreased with the increasing time of sampling and the lowest count 3,500,000/100 ml was observed in the last (60 minute) sample. (Appendix II, Figure 4).

Low levels (1 to 10/100 ml) of S. aureus were recorded in all stormwater samples (Appendix II).

During this event, salmonellae were detected in all (A to E) composite stormwater samples (Appendix II). Twelve isolates of Salmonella tennessee were obtained from these samples. Two isolates of the same Salmonella serotype were found in the Moore swab. Of the various media used, tetrathionate-brilliant green and selenite-brilliant green yielded 5 and 4 S. tennessee isolates, respectively.

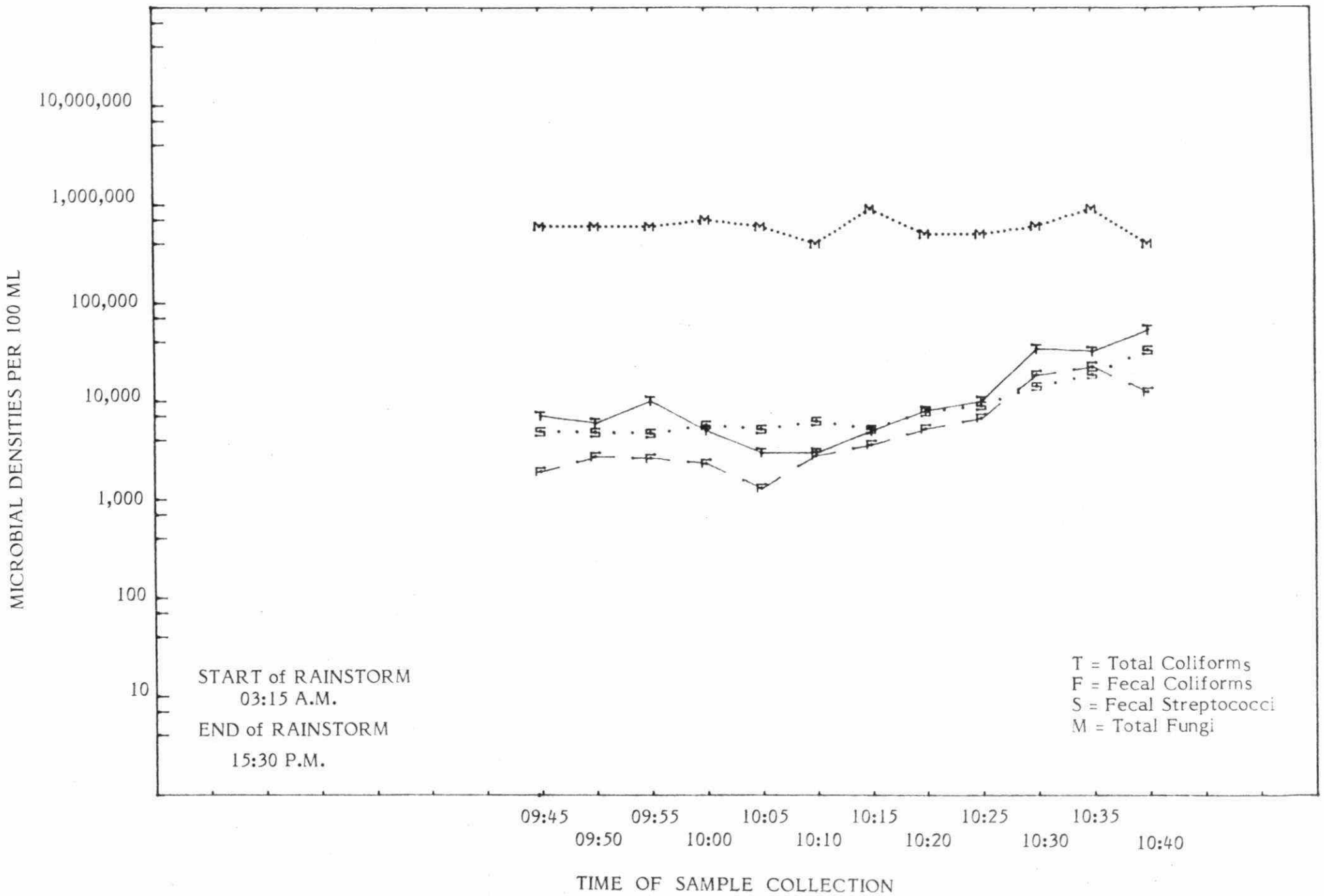
Storm of December 30, 1975

During this storm event which was of considerably longer duration (735 minutes) approximately 10.9 mm rainfall occurred. The first sample was obtained at 9:45 A.M. (390 minutes after the start of the storm), while the last sample was collected at 10:40 A.M. The storm event, however, continued till 3:30 P.M. (Appendix III).

The levels of indicator bacteria in all stormwater samples are presented in Figure 5. The densities of TC, FC and FS were generally low in 0 to 40 minute samples. However, elevated levels of these organisms were observed in samples collected at the 45 to 55 minute marks. Furthermore, the counts of all three indicator bacteria groups showed enormous variation. For example, TC values ranged from 7,000 to 53,000/100 ml, FC from 1,300 to 22,400/100 ml, and FS 4,700 to 33,000/100 ml.

Total fungal densities ranged from 400,000 to 900,000/100 ml. The highest counts were observed in the 30 and 50 minute samples, while the lowest level was recorded in the last (55 minute) sample (Appendix III, Figure 5).

FIGURE 5. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORMWATER RUNOFFS, December 30, 1975, Barrington Avenue (East York), Toronto



S. aureus was found in low numbers (10 to 20/100 ml) in only six of all the stormwater samples. P. aeruginosa was detected in all the samples with densities ranging from 130 to 630/100 ml (Appendix III). No salmonellae were isolated from either the Moore swab or any of the composite stormwater samples during the course of this storm event.

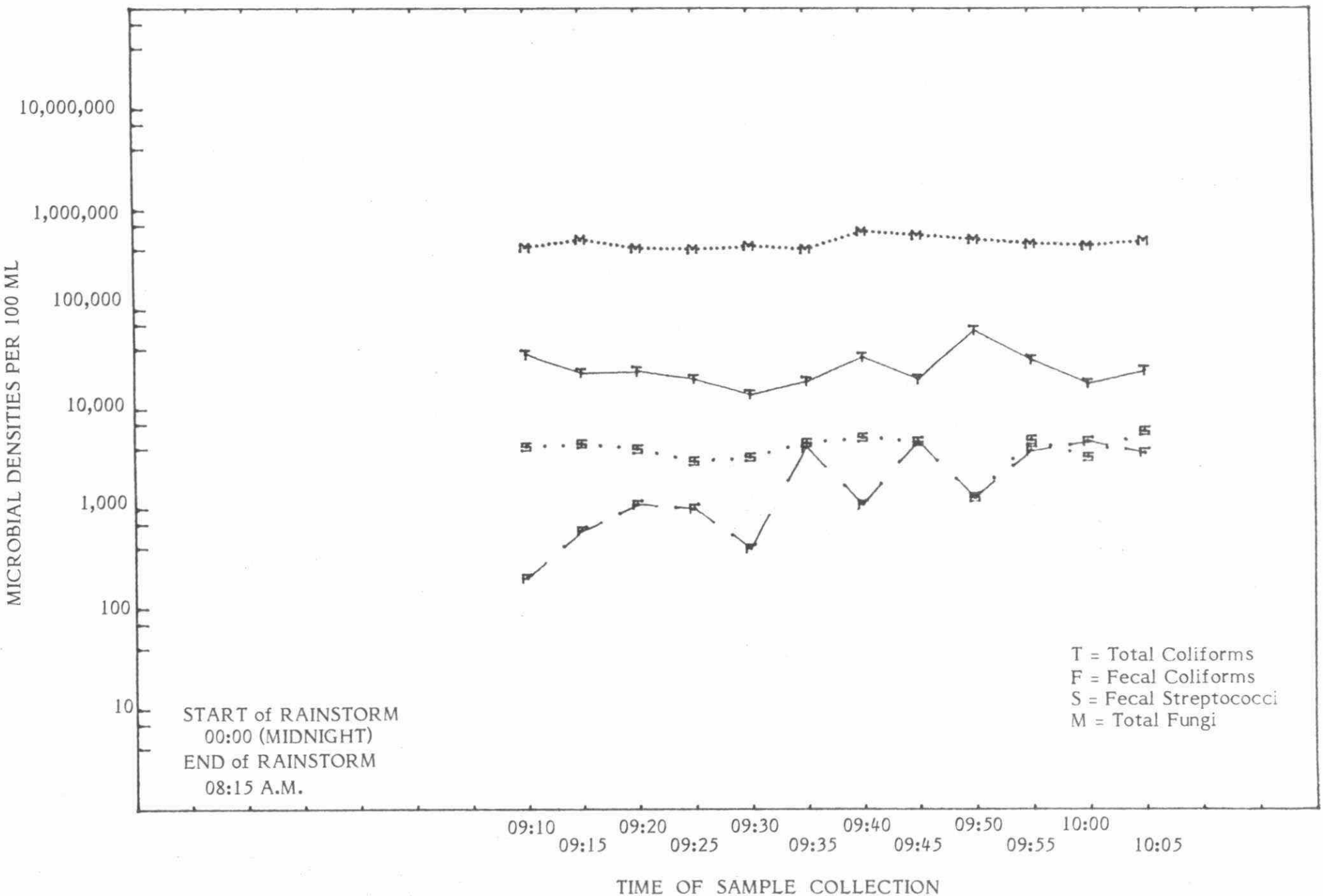
Storm of January 26, 1976

The storm lasted for 495 minutes and a total of 12.2 mm rain fell during this event. The sampling, however, was initated at 9:15 A.M. (approximately 55 minutes after the end of the storm) and contunued till 10:05 A.M. (Appendix IV). Since all samples were collected well after the rainfall has ended, they were not "typical" stormwater runoff samples and were mainly composed of snow-melt runoff.

The numbers and distribution of pollution indicator bacteria are illustrated in Figure 6. The densities of TC, FC and FS varied considerably throughout the entire sampling period and no consistent pattern of their distribution was established. As an example, TC levels (per 100 ml) ranged from 23,000 to 35,000 in 0 to 10 minute samples, from 14,000 to 33,000 in 15 to 35 minute samples, peaked (61,000) at the 40 minute mark, and varied from 18,000 to 31,000 in 45 to 55 minute samples. Likewise, FC numbers (per 100 ml) ranged from a low of 200 in the 0 minute sample to 47,000 in the 35 to 45 minute samples. Although, FS counts ranged from 3,000 to 6,000/100 ml, but exhibited lesser sample to sample variation as compared to the total and fecal coliform counts.

The densities of total fungi varied between 410,000 to 6,000,000/100 ml and showed little sample to sample variation. The maximum count was recorded in the 30 minute sample, while the minimum count was observed in the 10 minute sample (Appendix IV, Figure 6).

FIGURE 6. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORMWATER RUNOFFS, January 26, 1976, Barrington Avenue (East York), Toronto



As summarized in Appendix IV, S. aureaus was detected in all but two samples. The levels ranged from 10 to 100/100 ml. P. aeruginosa was isolated in low levels (10 to 20/100 ml) from only 50% of the samples. Salmonella typhimurium was isolated from the composite stormwater Sample A (one isolate), D (two isolates), and E (three isolates). However, no salmonellae were detected in the Moore swab. Three of the six S. typhimurium isolates were obtained from the selenite-XLD media combination during this storm event.

4.1.2 Sediment

Microbiological data obtained from the two sediment samples collected during dry weather conditions are given in Table 1. The densities of indicator bacteria were high, ranging from 10^4 to 10^8 per 100 ml. With one exception, the MF method produced higher TC, FC and FS counts than the MPN technique. As determined by the MF technique, the TC counts were 2,700,000 and 177,000,000; FC densities were 130,000 and 180,000; and FS levels were 90,000 and 190,000 in the two samples examined.

Using either the MPN or MF technique, P. aeruginosa densities could not be satisfactorily determined as appropriate sediment dilutions were not analyzed. In both samples, fungal densities were appreciably high, viz. 46,000 and 660,000 per gram sediment.

As shown in Table 2, no salmonellae were isolated from the September 29 sediment sample using either MF or Direct Inoculation (DI) techniques and the various enrichment-plating media combinations. However, in the November 4 sample, Salmonella typhimurium var. copenhagen was isolated from the tetrathionate-XLD combination when 25 grams sediment were directly inoculated into the enrichment medium. The other media combinations did not yield any Salmonella serotypes with this method of inoculation.

TABLE 1. MICROBIAL DENSITIES IN SEDIMENT SAMPLES COLLECTED FROM STORM SEWERS AT BARRINGTON AVENUE (EAST YORK), TORONTO AND WOODLAWN ROAD, GUELPH

Date	Site and Sample Collection Time	BACTERIAL PARAMETERS PER 100 ML								
		Total Coliform		Fecal Coliform		Fecal Streptococcus		Pseudomonas aeruginosa		Total Fungi[1]
		MF[2]	MPN[3]	MF	MPN	MF	MPN	MF	MPN	MF
September 29, 1975	Barrington Avenue 10:00	177,000,000	1,600,000	130,000	17,000	90,000	110,000	< 1,000	< 100	46,000
October 21, 1975	Woodlawn Road 14:00	260,000	1,600,000	20,000	49,000	10,000	79,000	< 1,000	< 100	33,000
November 4, 1975	Barrington Avenue 10:30	2,700,000	1,300,000	180,000	110,000	190,000	22,000	< 1,000	< 100	660,000

1 - Total Fungi CFU/G (Wet Weight Sediment).

2 - Membrane Filtration Technique.

3 - Most Probable Number Technique.

TABLE 2. ISOLATION OF SALMONELLA FROM SEDIMENT SAMPLES COLLECTED FROM STORM SEWERS AT BARRINGTON AVENUE (EAST YORK), TORONTO AND WOODLAWN ROAD, GUELPH

Date	Site and Sample Collection Time	SALMONELLA	
		Isolation Pathway *	Serotype
September 29, 1975	Barrington Avenue 10:00	MF (0.1, 0.01, 0.001, 0.0001 ml via serial sediment dilution) - TB, TX, SB, SX	NEG **
		DI (1,10, 50 ml of 1/10 sediment dilution) - TB, TX, SB, SX	NEG
October 21, 1975	Woodlawn Road 14:00	MF (0.1, 0.01, 0.001, 0.0001 via serial sediment dilution) - TB, TX, SB, SX	NEG
		DI (1, 10, 50 ml of 1/10 sediment dilution) - TB, TX, SB, SX	NEG
November 4, 1975	Barrington Avenue 10:30	DI (1, 50, 100 ml of 1/10 sediment dilution) - TB, TX, SB, SX	NEG NEG
		DI (25 g wet weight sediment) - TX	Salmonella typhimurium var. copenhagen
		- TB, SB, SX	NEG

* MF: Membrane Filtration Technique, DI: Direct Inoculation Technique.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

4.2 WOODLAWN ROAD, GUELPH

4.2.1 Stormwater Runoff

Storm of November 12, 1975

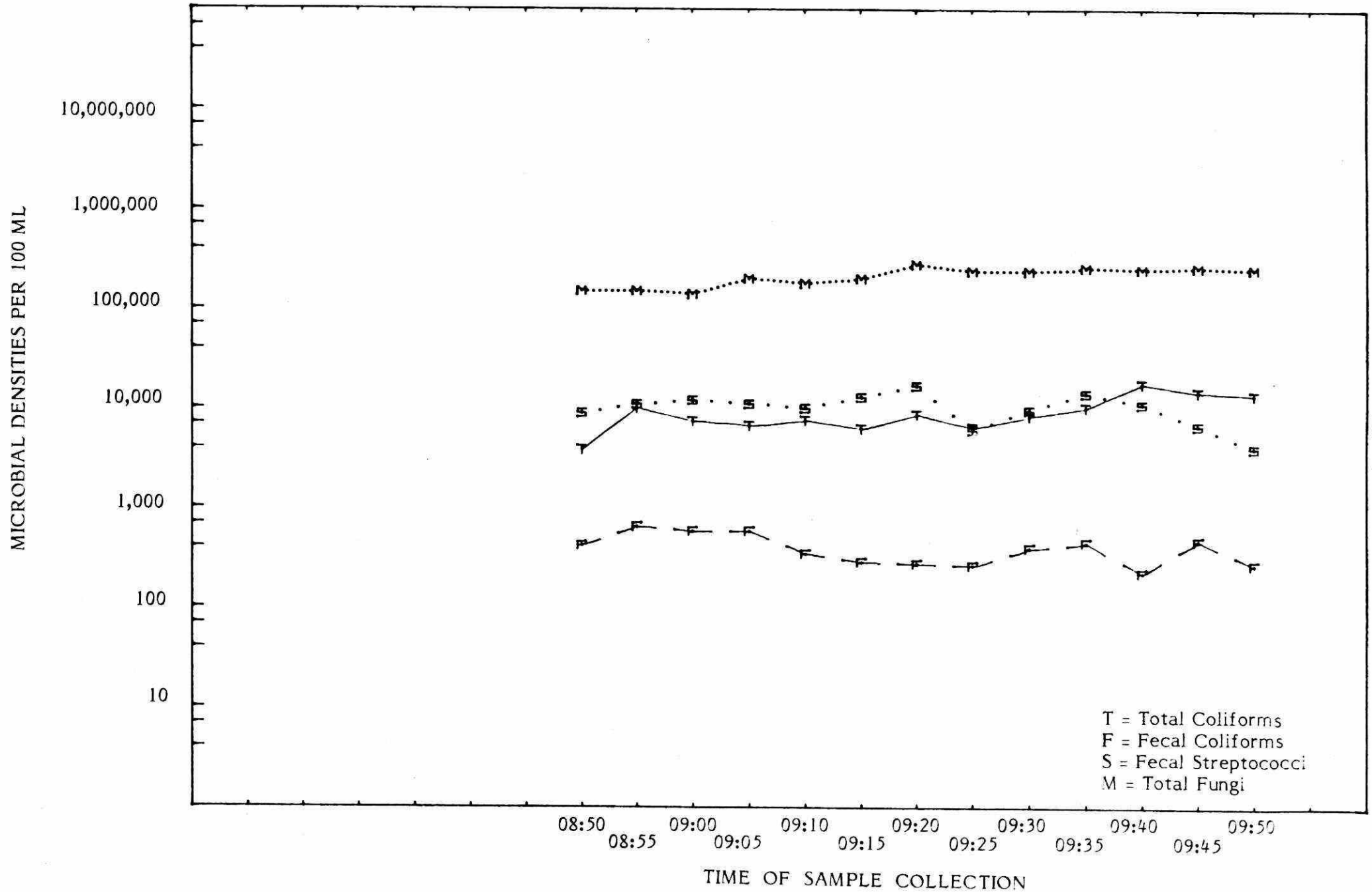
During this event no specific rainfall information was collected. The microbiological data obtained from the analysis of stormwater sample of this event are presented in Appendix V and Figure 7.

The TC levels (per 100 ml) varied considerably during the entire sampling period. For instance, in the first (0 minute) sample the TC count was 3,800; thereafter, densities increased and ranged from 6,200 to 10,200 in samples collected between the 5 and 45 minute marks. The peak (17,700) TC densities were observed in the 50 minute sample. The 55 and 60 minute samples yielded TC counts of 14,600 and 13,600, respectively. FC densities were generally low and varied from 220 to 630/100 ml exhibiting little sample to sample variation. FS levels showed an irregular pattern of distribution and the counts varied from 4,000 (60 minute sample) to 17,000 (30 minute sample) per 100 ml. Furthermore, in all samples, the FS densities were significantly (at least 20 times) higher than the FC levels.

Fungal densities (Appendix V, Figure 7) were generally low (ca. 140,000/100 ml) in the 0 to 10 minute samples. With one exception, fungal counts showed a relatively uniform distribution pattern in all of the other samples and the values ranged from 200,000 to 280,000/100 ml. The maximum level was recorded in the 30 minute sample.

S. aureus (1/100 ml) was found only in the 5 and 10 minute samples. P. aeruginosa was detected in all samples. In the 0 to 30 minute samples the densities were generally low (1 to 8/100 ml). However, slightly higher counts (28 to 32/100 ml) were recorded in the 50 to 60 minute samples (Appendix V).

FIGURE 7. DISTRIBUTION AND LEVELS OF POLLUTION INDICATOR BACTERIA AND TOTAL FUNGI IN STORMWATER RUNOFFS, November 12, 1975, Woodlawn Road, Guelph



No salmonellae were found in any of the stormwater samples. Three isolates of Salmonella haardt were obtained from the Moore swab exposed to the stormwater for 60 minutes during this event. Two of these isolates were obtained from the tetrathionate-BG combination, and selenite-BG yielded the third isolate.

4.2.2 Sediment

Microbiological data obtained from the only sediment sample collected during dry weather conditions are shown in Table 1. The levels of all pollution indicator bacteria examined were generally high and varied from 10^4 to 10^6 per 100 ml. The TC, FC and FS densities as determined by the MPN method (1,600,000, 49,000 and 79,000, respectively) were greater than the corresponding values obtained using the MF technique.

As inappropriate sediment dilutions were analyzed, no suitable counts of P. aeruginosa could be determined. In this sample, fungal densities were 33,000 per gram sediment. In addition, no salmonellae were detected in this sample using either the DI or MF procedures in conjunction with different combinations of enrichment and plating media (Table 2).

5.0 DISCUSSION

5.1 SUMMARY AND SYNTHESIS OF ALL MICROBIOLOGICAL DATA

During this investigation, stormwater samples were collected manually at least 15 minutes after the onset of a storm event. In one instance, although the storm event started at midnight, samples were collected in the morning, approximately one hour after the rainfall had ended. As a result, strictly speaking, no "zero" minute sample was collected during any of the storms monitored. The collection of such a sample was extremely difficult since, after the start of a storm, the field staff had to drive a substantial distance (requiring 10-20 minute travelling time) to reach the sampling site. In any event, for the purpose of

this investigation, the first sample collected was considered as the "zero" minute sample; thereafter, the sampling process continued for one hour during which additional samples were collected at five minute intervals.

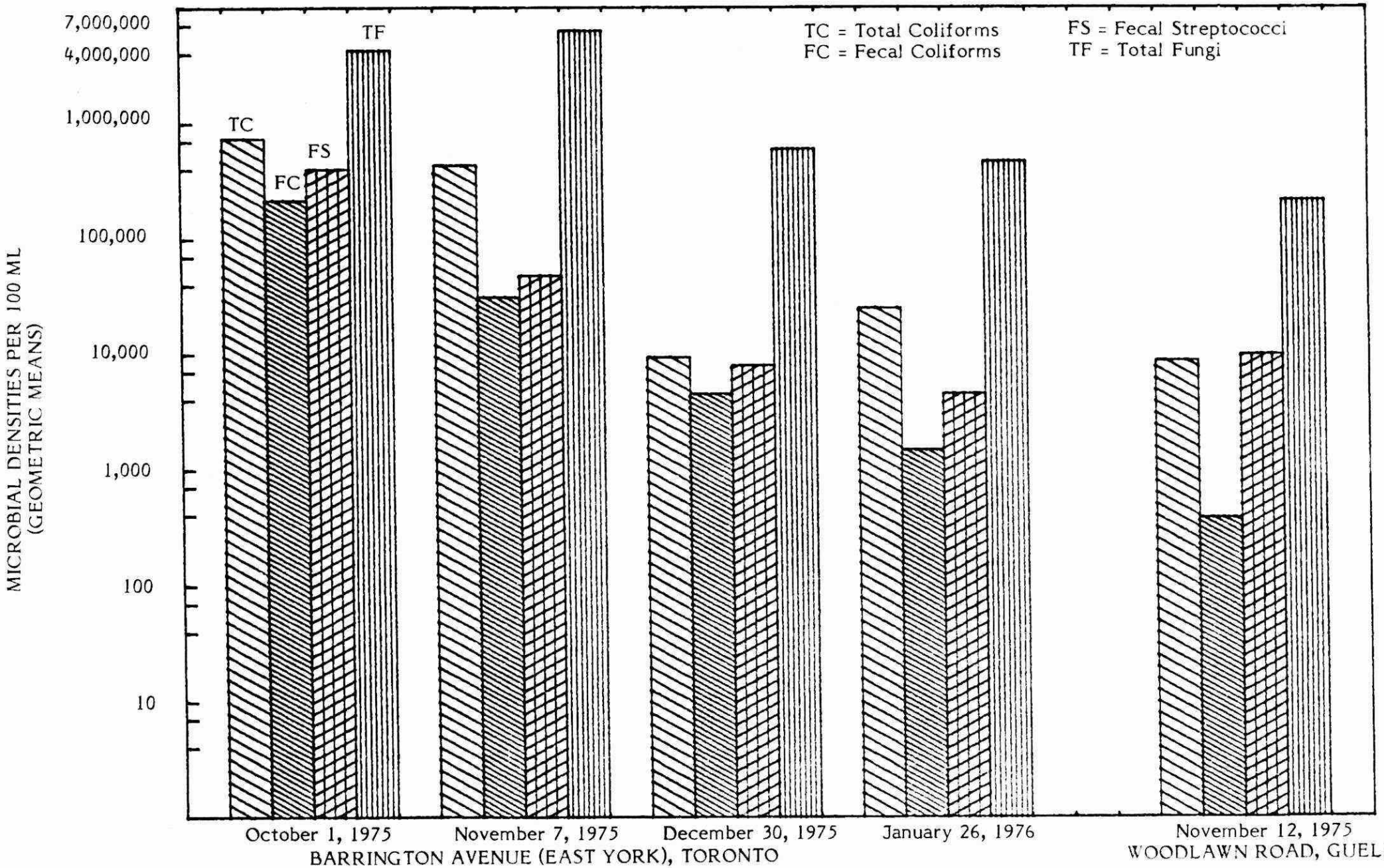
In addition to sample collection problems, the monitored rainstorms varied in intensity, duration and in the amount of total rainfall. As a result, no relationships or trends were evident with respect to the occurrence of maximum microbial populations in stormwater runoffs. For example, the peak microbial populations occurred either at the initial stages, middle or the tail-end of the sampling period of different storms monitored.

Although some variation was observed, in general, the initial counts (from 0 to 10 minute samples) of pollution indicator bacteria and total fungi were either at about the same level or slightly higher than the counts from 15 to 60 minute samples. This indicates that any effect of "Initial Flushing" on microbiological quality of the discharges was minimal during an individual storm.

All stormwater samples collected during the study period, from the two separate storm sewers, contained significant quantities of pollution indicator bacteria. However, no typical levels or patterns of distribution of these organisms could be established for individual storms. At the Barrington Avenue (East York, Toronto) site, the densities of TC varied from 3,000 to 1,190,000/100 ml. The TC geometric means for all the samples ranged from 9,330 to 730,000/100 ml (Figure 8). Fecal coliform levels ranged from 200 to 550,000/100 ml, while FC geometric means ranged from 1,460 to 216,000/100 ml (Figure 8). Fecal streptococcus densities were generally equal to or greater than the FC levels. The minimum and maximum FS counts were 3,000 and 620,000 per 100 ml, respectively. Geometric mean fecal streptococcus densities ranged from 4,520 to 402,000/100 ml (Figure 8).

In comparison, at the Woodlawn Road (Guelph) site, the densities of pollution indicator bacteria were lower than those obtained at the Toronto site. As summarized in Appendix V and Figure 8, the TC levels varied from 3,800 to 17,700,

FIGURE 8. MICROBIAL POPULATIONS IN DISCHARGES FROM FIVE STORM EVENTS AT THE TWO URBAN SEPARE STORM SEWERS



with a geometric mean of 8,560 per 100 ml. Fecal Coliform counts ranged from 220 to 630 per 100 ml with a geometric mean of 370. Fecal streptococcus densities were greater than FC levels and ranged from 6,100 to 17,000 per 100 ml, the geometric mean value being 9,730.

Several other investigators (2, 5, 6, 10) reported TC, FC and FS densities in stormwaters from separate storm sewer systems similar to those found in the present study. As an example, Weibel et al (2) found that in 50% of all stormwater samples collected from a separate storm sewer, the levels of TC, FC and FS were greater than 58,000, 10,900 and 20,500 per 100 ml, respectively. Furthermore, Geldreich et al (10) reported seasonal variation (autumn densities higher than winter) in the populations of indicator bacteria in stormwaters collected from suburban areas. Benzie and Courchaine (6) found that the levels of coliforms were lower in stormwater runoffs during winter and early spring than for the warmer weather periods. Similar results were obtained in the present study as the TC, FC and FS densities were considerably greater (at the Toronto site) in samples obtained during October-November than those collected in December-January period.

Fungal population estimates varied between 400,000 and 12,000,000 per 100 ml in stormwaters collected from the Toronto site. The geometric mean values for all samples ranged from 460,000 to 6,240,000 (Figure 8). In discharges from the Guelph storm sewer, the minimum and maximum fungal densities were 140,000 and 280,000 with a geometric mean of 210,000 per 100 ml (Figure 8).

With respect to opportunistic pathogenic bacteria, S. aureus was infrequently isolated from stormwaters at both sites. The densities were generally low and, with few exceptions, ranged from 1 to 40 per 100 ml. The presence of P. aeruginosa was examined in only 50% of the samples. It was detected in all samples examined and the densities (10 to 630/100 ml) at the Toronto site were greater than the Guelph storm sewer where the levels varied between 1 and 32 per 100 ml.

During this investigation, twenty-eight isolates of Salmonella were isolated from stormwaters at both the Guelph and Toronto storm sewers (Table 3). These isolates belonged to four different serotypes including S. saint-paul, S. haardt, S. tennessee and S. typhimurium. Most of the isolates (about 90%) were obtained from the Toronto storm sewer where salmonellae were detected in as little as 10 ml of stormwater sample (Appendix I). Salmonellae were also isolated from 50, 100 and 300 ml aliquots of either separate or composite stormwater samples and from a Moore swab (Appendix I, II and III). At the Guelph site, Salmonella was found only in the Moore swab and not in the sediment sample.

As with the pollution indicator bacteria, no definite pattern of occurrence and distribution of salmonellae was evident in this study. These organisms were found, though at different time intervals, in all samples collected during the sixty minute sampling period. This indicates that pathogenic bacteria, like Salmonella, are probably discharged throughout the entire span of a storm event.

Evans et al (9) reported the presence of Salmonella in a stormwater sample, collected from an urban separate storm sewer, which contained significant quantities of fecal pollution indicator bacteria. They estimated that one isolate of Salmonella was detected for every 100 fecal coliforms in that sample. In this study no relationships were evident between the incidence of salmonellae and the levels and peaks of indicator bacteria in stormwaters examined. It must be pointed out, however, that it is generally assumed that the chances of Salmonella isolation are greater from those samples which contained large numbers of fecal coliforms than from those with low fecal coliform densities (34, 35, 36). The relationships between pathogenic microorganisms and indicator organisms have also been reviewed recently by several workers in the "Proceedings of Workshop on Microorganisms in Urban Stormwater" (37) and in a comprehensive report on "Microorganisms in Urban Stormwater" (58).

TABLE 3. SUMMARY OF SALMONELLA ISOLATION FROM STORMWATER RUNOFF AND SEDIMENT SAMPLES COLLECTED FROM THE TWO SEPARATE STORM SEWERS

SITE	SALMONELLA SEROTYPE	NUMBER OF ISOLATES
Barrington Avenue (East York, Toronto)	Sediment	
	Salmonella typhimurium var. *copenhagen*	1
	Stormwater Runoff	
	S. saint-paul	1
	S. tennessee	14
	S. typhimurium	6
	S. typhimurium var. *copenhagen*	3
Woodlawn Road (Guelph)	Stormwater Runoff (Moore Swab)	
	S. haardt	3
TOTAL:		28

Sediment samples collected during dry weather conditions from these two storm sewers yielded large quantities of fecal indicator bacteria. Their levels were either equal to or greater than those in stormwater runoffs. Fungal densities were also substantially higher in sediments. Salmonella was found in only one sediment sample obtained from the Toronto storm sewer. The serotype S. typhimurium var. copenhagen was also found in stormwaters at this site.

5.2 SOURCES OF MICROBIAL POLLUTION IN STORMWATER RUNOFF AND ASSOCIATED POTENTIAL HEALTH HAZARDS

It may be assumed that there is no apparent source of contamination with domestic waste discharges in both the Guelph and Toronto residential storm sewers as they are separate stormwater systems. The microbiological data from these storm sewers, however, demonstrated that stormwater discharges contained substantial "fecal" pollution. The levels of TC, FC and FS were strikingly high throughout the sampling period of 60 minutes and many times reached densities found in untreated domestic sanitary discharges. The high concentration of indicator bacteria was accompanied by the presence of certain pathogenic and potentially pathogenic bacteria in these stormwater runoffs.

Geldreich and Kenner (38) proposed that the sources of fecal bacterial pollution may be differentiated by using a fecal coliform to fecal streptococcus (FC:FS) ratio. It was suggested that if the FC:FS ratio is greater than 4.0, the source is likely of human origin; while if the ratio is less than 0.7 then the source is probably of non-human origin. The FC:FS ratios between 0.7 and 4.0 have been considered as "indeterminate" as to source; they may be from mixed, old or atypical sources. Although this concept has received general acceptance and has been widely used, several workers (38, 39, 40, 41, 42, 43) emphasized that the

FC:FS ratio must be employed with some degree of caution since it is time dependent. They pointed out that once discharged into receiving waters, the differential die-off rates of these organisms and diverse environmental factors may alter their interrelationship to such an extent so as to render the FC:FS ratio to be of limited or no significance in determining the source of bacterial contamination.

The results obtained in this study indicate that a relationship between fecal coliforms and fecal streptococci does exist within certain limits of interpretation. At the Guelph site, fecal contamination is primarily of non-human origin as fecal streptococcus densities were significantly greater than fecal coliforms. The data from the Toronto storm sewer are rather inconclusive; however, FS levels, which are generally equal to or greater than FC counts, would indicate that bacterial contamination is predominately of non-human origin. It is possible that, at this site, a portion of fecal contamination may have originated from the unknown entry of sanitary discharges through illegal cross-connections. Previous investigators (2, 5, 6, 9, 10, 38, 58) reported similar findings and demonstrated that fecal pollution in the separate storm sewer systems is predominately of non-human origin.

Fecal contamination in these two separate storm sewer systems, situated in residential areas, appears to be derived initially from feces deposited by pets*, particularly cats and dogs, rodents and birds. In addition, runoff from soil previously contaminated with animal waste may contribute fluctuacting levels of fecal contamination to drainage water. During periods of rainfall, some of the indicator bacteria associated with a variety of vegetation (44) may also enter the stormwater as part of overland-wash. Furthermore, it has been suggested that both coliforms and non-coliforms proliferate (characterized as after growths) in

* According to a conservative estimate made by The Toronto Humane Society "there are about 4,000 dogs and 38,000 cats in the city". In Canada, a cross-country survey in 1975 showed "that in cities of 100,000 or more 25% of the homes had one or more dogs and that 24% of the homes had one or more cats" (The Globe and Mail, Toronto, May 11, 1977).

sediments and soil and as such may be of little sanitary significance (17, 45). Nevertheless, the strikingly high levels of microbial populations observed in sediments in this study do indicate that storm sewer sediments constitute a reservoir of indicator bacteria as well as pathogens that may eventually find their way into stormwater runoffs.

The remarkably high concentration of fecal pollution indicator bacteria in discharges from these two residential urban separate storm sewers indicate possible health hazards. Water bodies receiving untreated stormwaters are not suitable for bathing, swimming and general recreation because such discharges are potential focci of human and animal infections.

P. aeruginosa (an opportunistic pathogen) has become a common cause of nosocomial infections (46, 47) and is regarded as a major etiological agent of otitis externa among swimmers (47, 48). The presence of P. aeruginosa in stormwaters at both sites also constitutes an elevated risk to public health.

The detection of salmonellae in both stormwater and sediments in this study further substantiates the existence of potential health hazards in stormwater runoffs. Of the Salmonella serotypes isolated, S. typhimurium and S. saint-paul were among the 10 most common serotypes isolated from humans in Denmark and in the United States (49). S. typhimurium was also one of the 13 strains most frequently isolated from both diseased and healthy farm animals (49). Moreover, all four serotypes obtained in this study have also been isolated from humans in Canada during the past several years. For example, in Ontario in 1975 (Personal Communications from Dr. A. Evans, Community Health Protection Branch, Ontario Ministry of Health, Toronto), S. typhimurium and S. thyphimurium var. copenhagen were most frequently isolated; while the incidence of S. saint-paul, S. tennessee and S. haardt was less common. S. typhimurium var. copenhagen was involved in one outbreak which accounted for 46 cases of illness.

During this investigation, substantially high populations of fungi (including both molds and yeasts) were found in sediments and stormwater discharges at the two sites examined. Although the procedures used in this study did not delineate the saprophytic and pathogenic fungi, their presence in large quantities demonstrated that stormwater runoffs may contribute yet another group of potential pathogens to the recipient waters. Bergen and Wagner-Merner (50) recently reported the occurrence of potentially pathogenic fungi from several beaches in the Tamp Bay area.

5.3 URBAN STORMWATER RUNOFF POLLUTION CONTROL

In recent years urban stormwater runoff has been recognized as a significant source of a wide variety of pollutants to surface waters. The information provided in this report further substantiates that urban stormwater discharges are a major source of intermittent microbial pollution to receiving water. The results of various other studies and general awareness of harmful effects from urban stormwater runoffs have motivated the development and implementation of programs to control water pollution from such nonpoint sources. All of the methods proposed and designed to control pollution from stormwater runoffs include different types of storage of stormwaters and their subsequent treatment by various means. Several investigators (11, 15, 20, 23, 52-57) have provided comprehensive reviews of different methods employed in planning, management and control of urban nonpoint source pollution.

Furthermore, in order to prevent and control microbial pollution in stormwater runoffs, certain remedial measures should be applied at sources of such contamination. These may include: (i) pet population control, (ii) efficient and effective removal of animal waste from streets, (iii) sanitation and effective

garbage control to discourage increased rodent habitation of storm sewers, and (iv) improved maintenance of storm sewers to avoid excessive accumulation of sediments which act as reservoirs of microbial contamination. The remedial measures should also include education and awareness programs to better inform the general public about the existence, significance, sources and control of urban pollution from nonpoint sources.

5.4 COMMENTS ON METHODOLOGIES USED IN THIS STUDY

5.4.1 Sample Collection

Problems were encountered in manual sample collection of stormwaters, particularly in obtaining "zero" minute samples since the technician had to drive to the site after the initiation of a storm event. In addition, during those events which started during the night, samples were collected in the morning when the storm had already been in progress for several hours.

An alternative would be automatic sampling which was not attempted in the present study. However, samples collected by automatic means are suitable for microbiological examination only if they are collected in sterile refrigerated containers. Such an automatic sampler is not available at the present time.

In future studies, manual sample collection should be carried out by a local resident who can immediately reach the site following the onset of the storm. Of course, this arrangement will require some initial training of such a person for the collection of microbiological samples.

5.4.2 Pollution Indicator Bacteria

The MF techniques and procedures used for the isolation and enumeration of TC, FC and FS from stormwater runoff samples were found to be satisfactory and provided meaningful estimates of populations of these organisms.

Since problems were encountered in analyzing sediments by the MF technique, the most probable number (MPN) technique should be used in future studies for the enumeration of pollution indicator bacteria.

5.4.3 Pseudomonas aeruginosa

The use of MF technique and the mPA medium were satisfactory for the determination of P. aeruginosa densities in water samples. The levels in sediments, however, should be ascertained by the MPN procedure using 3 to 5 dilution/tube series.

5.5.4 Coagulase-positive Staphylococci (Staphylococcus aureus)

The method used for the determination of S. aureus levels in water samples proved to be unsatisfactory. The prcedure is lengthy, tedious, requires several confirmatory steps and as such is not appropriate for routine analysis. In addition, problems were encountered during the enumeration step due to the presence of false positive and false negative colonies. As a result the medium and procedure are not suitable for S. aureus isolation from environmental samples. Further work is required to either modify this medium or develop a new method for the enumeration of S. aureaus from natural waters.

5.4.5 Total Fungi (Molds and Yeasts)

The spread plate technique and STMEA medium were found to be suitable for the determination of fungal populations both in water and sediments. The MF technique can also be used for the enumeration of fungi in natural waters as described previously (32).

5.5.5 Salmonella

Since stormwater runoffs contained remarkably high levels of microbial populations, salmonellae were isolated from as little as 10 ml sample aliquots. The maximum sample volume which yielded Salmonella was 300 ml. However, for the detection of salmonellae in waters from a less polluted

environment, the use of larger volumes is recommended. This can be achieved by filtering sample aliquots ranging from 500 to 1000 ml through 3 or 4 prefilters (similar to those used in this study), which then can be placed in the enrichment broth. The use of Moore swabs was also satisfactory for Salmonella isolation and its use, where possible, is highly recommended for any future studies of this nature.

Although the scheme used here for the detection of Salmonella requires 5 days for positive identification, it was reliable and very productive. Once the MacConkey agar and ONPG-PA agar screening test were completed (on the 3rd day), only a small number of non-salmonellae were eliminated on the basis of subsequent biochemical tests. With respect to the Salmonella isolation pathway, 24 h enrichment at 41.5°C was fairly productive, however, extended (48 h) incubation may enhance recovery of Salmonella. Whereas both tetrathionate and selenite broths provided almost equal numbers (15 and 13, respectively) of Salmonella isolates, the BG plating medium yielded 19 isolates compared to 9 on XLD agar. The tetrathionate-BG Combination was the most productive and produced 11 (39.3%) isolates, this was followed by selenite-BG with 8 (28.6%) isolates. Im comparison, the tetrathionate-XLD and selenite-XLD combinations were less productive and yielded 4 (14.3%) and 5 (17.8%) isolates, respectively.

The use of sediment suspension-dilution was unsuccessful for the detection of salmonellae in sediments. Salmonella was isolated only once when sediments (25 g) were inoculated directly into the enrichment broth. This procedure is highly recommended for any future studies of salmonellae isolation from sediments.

The method used in this study provided only qualitative determination of salmonellae; in future, attempts should be made to develop procedures for quantitative isolation of Salmonella. Furthermore, additional research should be conducted, using several other enrichment broths and plating agars, to select one most productive combination for Salmonella islation from natural waters. The use of only one enrichment broth and plating medium will decrease the test cost and workload considerably, and may also permit extended (48 h) enrichment.

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A P P E N D I C E S

APPENDIX I. LEVLES AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus aureus	TOTAL FUNGI (CFU†/100 ML)
October 1, 1975	10:20 - 10:50	2.3 mm	10:35	0	380,000	120,000	400,000	NA*	< 10	3,000,000
			10:40	5	930,000	340,000	420,000	NA	< 10	3,000,000
	(30)		10:45	10	1,030,000	360,000	620,000	NA	< 10	8,000,000
			10:50	15	740,000	260,000	470,000	NA	< 10	9,000,000
			10:55	20	1,020,000	150,000	550,000	NA	30	7,000,000
			11:00	25	720,000	80,000	410,000	NA	20	4,000,000
			11:05	30	750,000	360,000	340,000	NA	OBS**	4,000,000
			11:10	35	1,190,000	420,000	420,000	NA	40	5,000,000
			11:15	40	810,000	300,000	440,000	NA	40	3,000,000
			11:20	45	560,000	170,000	230,000	NA	20	3,000,000
			11:25	50	530,000	210,000	310,000	NA	16	5,000,000
			11:30	55	540,000	140,000	360,000	NA	12	2,000,000

* NA: Not Analyzed.

** OBS: Obscured.

† CFU: Colony- forming units.

APPENDIX I. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
10:35	0	-	10, 50, 100 - TB, TX, SB, SX	NEG**
10:40	5	-	——— " ———	NEG
10:45	10	-	——— " ———	NEG
10:50	15	-	10 - TB	Salmonella saint-paul
			50, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
10:55	20	-	10 - TB	S. typhimurium var. copenhagen
			50, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
11:00	25		10 - TB	S. typhimurium var. copenhagen
			50, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
11:05	30	-	50 - TB	S. typhimurium var. copenhagen
			10, 100 - TB, TX	NEG
			10, 50, 100 - SB, SX	NEG
11:10	35	-	10, 50, 100 - TB, TX, SB, SX	NEG
11:15	40	-	——— " ———	NEG
11:20	45	-	——— " ———	NEG
11:25	50	-	——— " ———	NEG
11:30	55	-	——— " ———	NEG
11:35	60	Moore Swab	Strips 1, 2, 3 - TB, TX	NEG
			4, 5, - SB, SX	NEG

* Sample Volume Analyzed: 10, 50, 100 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± h.

** NEG: Negative.

APPENDIX II. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus aureus	TOTAL FUNGI (CFU/100 ML)
November 7, 1975	15:40 - 16:30 (50)	1.0 mm	15:55	0	790,000	550,000	240,000	NA*	1	12,000,000
			16:00	5	270,000	140,000	120,000	NA	4	8,000,000
			16:05	10	240,000	120,000	60,000	NA	1	8,000,000
			16:10	15	360,000	10,000	50,000	NA	4	6,000,000
			16:15	20	620,000	10,000	30,000	NA	10	6,000,000
			16:20	25	380,000	10,000	70,000	NA	4	6,000,000
			16:25	30	720,000	50,000	60,000	NA	4	7,000,000
			16:30	35	330,000	10,000	40,000	NA	4	6,000,000
			16:35	40	620,000	10,000	10,000	NA	4	6,000,000
			16:40	45	580,000	50,000	60,000	NA	4	6,000,000
			16:45	50	390,000	10,000	10,000	NA	4	5,000,000
			16:50	55	320,000	30,000	70,000	NA	4	5,000,000
			16:55	60	430,000	40,000	40,000	NA	1	3,500,000

* NA: Not Analyzed.

APPENDIX II. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
15:55	0	A	100 - TB	Salmonella tennessee
16:00	5		100 - SB	S tennessee
16:05	10		100 - TX, SX	NEG **
			50, 300 - TB, TX, SB, SX	NEG
16:10	15	B	100 - SB	S. tennessee
16:15	20		100 - TB, TX, SX	NEG
			50, 150 - TB, TX, SB, SX	NEG
16:20	25	C	100 - TX	S. tennessee
16:25	30		150 - TX	S. tennessee
			100, 150 - TB, SB, SX	NEG
			50 - TB, TX, SB, SX	NEG
16:30	35	D	100 - SB	S. tennessee
16:35	40		300 - TB	S. tennessee
16:40	45		300 - SX	S. tennessee
			100 - TB, TX, SX	NEG
			300 - TX, SB	NEG
			50 - TB, TX, SB, SX	NEG
16:45	50	E	300 - TB	S. tennessee
16:50	55		300 - TX	S. tennessee
16:55	60		300 - SB	S. tennessee
			300 - SX	S. tennessee
			50, 100 - TB, TX, SB, SX	NEG
16:55	60	Moore Swab	Strips 1, 2, 3 - TB	S. tennessee
			- TX	NEG
			4, 5, - SB	S. tennessee
			- SX	NEG

* Sample Volume Analyzed: 50, 100, 150, 300 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

APPENDIX III. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus areua	TOTAL FUNGI (CFU/100 ML)
December 30, 1975	3:15 -	10.9 mm	9:45	0	7,000	1,900	4,900	330	0	600,000
	15:30*		9:50	5	6,000	2,700	4,800	190	10	600,000
	(735)		9:55	10	10,000	2,600	4,700	270	10	600,000
			10:00	15	5,000	2,300	5,300	130	10	700,000
			10:05	20	3,000	1,300	5,200	290	20	600,000
			10:10	25	3,000	2,800	6,300	290	20	400,000
			10:15	30	5,000	3,600	5,200	240	0	900,000
			10:20	35	8,000	5,200	7,800	630	20	500,000
			10:25	40	10,000	6,700	9,000	350	0	500,000
			10:30	45	34,000	18,500	14,200	520	0	600,000
			10:35	50	32,000	22,400	18,700	320	0	900,000
			10:40	55	53,000	12,400	33,000	330	0	400,000

* Samples were taken during the most intense period of rainfall, and 2.5 mm rain fell during the sampling period.

APPENDIX III. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
9:45 9:50 9:55	0 5 10	A	50, 100, 300 - TB, TX, SB, SX	NEG **
10:00 10:05	15 20	B	50, 100, 300 - TB, TX, SB, SX	NEG
10:10 10:15	25 30	C	50, 100, 150 - TB, TX, SB, SX	NEG
10:20 10:25	35 40	D	50, 100, 150 - TB, TX, SB, SX	NEG
10:30 10:35 10:40	45 50 55	E	50, 100, 300 - TB, TX, SB, SX	NEG
10:45	60	Moore Swab	Strips 1, 2, 3 - TB, TX 4, 5 - SB, SX	NEG NEG

* Sample Volume Analyzed: 50, 100, 150, 300 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

APPENDIX IV. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM BARRINGTON AVENUE (EAST YORK) STORM SEWER, TORONTO

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus aureus	TOTAL FUNGI (CFU/100 ML)
January 26, 1976	00:00 -	12.2 mm	9:10	0	35,000	200	4,200	10	10	420,000
	8:15*		9:15	5	23,000	600	4,500	0	20	500,000
	(495)		9:20	10	24,000	1,100	4,000	0	10	410,000
			9:25	15	20,000	1,000	3,000	0	20	400,000
			9:30	20	14,000	400	3,300	0	10	430,000
			9:35	25	19,000	4,100	4,600	20	100	400,000
			9:40	30	33,000	1,100	5,200	10	0	600,000
			9:45	35	20,000	4,700	6,600	0	100	550,000
			9:50	40	61,000	1,300	6,300	10	0	500,000
			9:55	45	31,000	3,800	4,900	0	0	450,000
			10:00	50	18,000	4,700	3,300	10	100	430,000
			10:05	55	24,000	3,600	6,000	10	10	480,000

* Samples were taken after rainfall ended and were mainly composed of melting-snow runoff.

APPENDIX IV. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
9:10	0	A	50 - TB	Salmonella typhimurium
9:15	5		50 - TX, SB, SX	NEG **
9:20	10		100, 300 - TB, TX, SB, SX	NEG
9:25	15	B	50, 100, 150 - TB, TX, SB, SX	NEG
9:30	20			
9:35	25	C	50, 100, 150 - TB, TX, SB, SX	NEG
9:40	30			
9:45	35	D	50 - SB	S. typhimurium
9:50	40		50 - SX	S. typhimurium
			50 - TB, TX	NEG
			100, 150, TB, TX, SB, SX	NEG
9:55	45	E	50 - SX	S. typhimurium
10:00	50		100 - SB	S. typhimurium
10:05	55		100 - SX	S. typhimurium
			50 - TB, TX, SB	NEG
			100 - TB, TX	NEG
			300 - TB, TX, SB, SX	NEG
10:10	60	Moore Swab	Strips 1, 2, 3 - TB, TX	NEG
			4, 5 - SB, SX	NEG

* Sample Volume Analyzed: 50, 100, 300 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.

APPENDIX V. LEVELS AND DISTRIBUTION OF MICROORGANISMS IN STORMWATER RUNOFF FROM WOODLAWN ROAD STORM SEWER, GUELPH

A. POLLUTION INDICATOR BACTERIA, PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, TOTAL FUNGI

STORM			SAMPLE COLLECTION		BACTERIAL PARAMETERS PER 100 ML					
Date	Duration (Minutes)	Amount of Rainfall	Time	Accumulative Time (Minutes)	Total Coliform	Fecal Coliform	Fecal Streptococcus	Pseudomonas aeruginosa	Staphylococcus areus	TOTAL FUNGI (CFU/100 ML)
November 12, 1975	NA*	NA*	8:50	0	3,800	410	8,900	4	0	150,000
			8:55	5	9,900	630	11,000	8	1	150,000
			9:00	10	7,300	570	12,000	4	1	140,000
			9:05	15	6,600	570	11,000	1	0	200,000
			9:10	20	7,500	340	10,000	4	0	180,000
			9:15	25	6,200	280	13,000	4	0	200,000
			9:20	30	8,600	270	17,000	1	0	280,000
			9:25	35	6,400	260	6,100	24	0	240,000
			9:30	40	8,300	390	9,600	4	0	240,000
			9:35	45	10,200	440	14,200	20	0	260,000
			9:40	50	17,700	220	11,000	28	0	250,000
			9:45	55	14,600	460	6,700	32	0	260,000
			9:50	60	13,600	264	4,000	28	0	250,000

* NA: Not Available.

APPENDIX V. (Continued)

B. SALMONELLA

SAMPLE COLLECTION			SALMONELLA	
Time	Accumulative Time (Minutes)	Composite Sample	Isolation Pathway *	Serotype
8:50	0	A	400 - TB, TX, SB, SX	NEG **
8:55	5			
9:00	10			
9:05	15	B	250 - TB, TX, SB, SX	NEG
9:10	20			
9:15	25	C	200 - TB, TX, SB, SX	NEG
9:20	30			
9:25	35	D	250 - TB, TX, SB, SX	NEG
9:30	40			
9:35	45			
9:40	50	E	350 - TB, TX, SB, SX	NEG
9:45	55			
9:50	60			
9:50	60	Moore Swab	Strips 1 - TB, TX	NEG
			2 - TB	Salmonella haardt
			3 - TB	S. haardt
			2, 3 - TX	NEG
			4 - SB, SX	NEG
			5 - SB	S. haardt
			5 - SX	NEG

* Sample Volume Analyzed: 200, 250, 350, 400 ml.

Enrichment Broths, Temperature, Incubation: T = Tetrathionate, 41.5°C, 20 ± 2 h; S = Selenite, 41.5°C, 20 ± 2 h.

Plating Agars, Temperature, Incubation: B = Brilliant Green Agar, 41.5°C, 20 ± 2 h; X = XLD Agar, 41.5°C, 20 ± 2 h.

** NEG: Negative.